

Magnetic Microcapsules As Novel Biomonitor of Cross-Linking Agents and Diet-Dependent Reactive Oxygen Species in the Human Gastrointestinal Tract

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Six healthy, scientifically informed human volunteers were given ¹⁴C-labeled polyethyleneimine (PEI) microcapsules by mouth. Fecal ¹⁴C recovery was inversely related to mean gut transit time ($r = -0.66$), and the extent of cross-linking between the membrane and core PEI was inversely related to total fecal output ($r = -0.81$). Cross-linking of PEI microcapsules may be a biomonitor of endogenous cross-linking agents within the human gastrointestinal tract. Extensive loss of [¹⁴C]CH₃ label occurred from the microcapsules during human transit and in *in vitro* fermentations with human fecal flora. A mechanism whereby reactive oxygen species could arise in the iron-rich core of these microcapsules, leading to loss of [¹⁴C]CH₃ label, is proposed.

Introduction

The importance of diet (1) and the high contribution from gastrointestinal (GI) sites to human cancer risk (2), have prompted us to devise a biomonitoring technique for the GI cavity (3). This system consists of an oral dose of aqueous suspension of millions of magnetically recoverable, semipermeable microcapsules with a tough nylon membrane enclosing a polyamine, polyethyleneimine (PEI). Previous studies in rodents have shown that PEI microcapsules trapped alkylating agents (4), *N*-nitrosating agents (5), mutagens/carcinogens having planar molecular structure (6,7), and bifunctional alkylating (cross-linking) agents (8). For the first time, we have used these microcapsules as biomonitor in healthy free-living humans.

Methods

Semipermeable, magnetic microcapsules with a membrane consisting of polyhexamethylenediamineterephthalamide grafted on to polyethyleneimine (M.W. 50,000) were prepared under modified conditions to that reported previously (3) so as to

have a larger diameter and were sieved several times through 25- μ m and then 50- μ m sieves in order to remove smaller microcapsules. A 50-mL suspension in ethanol was treated with [¹⁴C]CH₃I in ethanol (100 μ Ci, 56 mCi/mmol) overnight with shaking at 37°C, washed several times, and then diluted to 26.4 kBq/mL, measured by suspension in scintillation fluid. Full details will be reported elsewhere (9).

One milliliter of the ¹⁴C-labeled microcapsules was diluted with 3 mL ethanol, transferred to gelatin capsules, and administered to six healthy, nonsmoking, scientifically informed subjects (five men, one woman, age range 26-59 years), together with 200 radio-opaque markers (ROM) (10), also in gelatin capsules. Baseline spot urines and stool specimens were collected by all the subjects immediately before administration of the microcapsules. After taking the microcapsules, subjects collected all fecal specimens, which were X-rayed to count the ROM, until all ROM had been recovered, and for a further 3 days. Urine samples were collected for 4-6 days and were checked for their completeness using *p*-aminobenzoic acid (PABA) (11).

Fecal samples were individually digested with 10% aqueous Tween solution for 1 hr, and aliquots were removed for ¹⁴C counting after sample oxidation. Microcapsules were removed magnetically from the remaining fecal homogenate and stored in ethanol at room temperature until analysis.

For estimation of cross-linking, a 100 μ L aliquot of microcapsules was diluted with water to 1.0 mL and the membranes rup-

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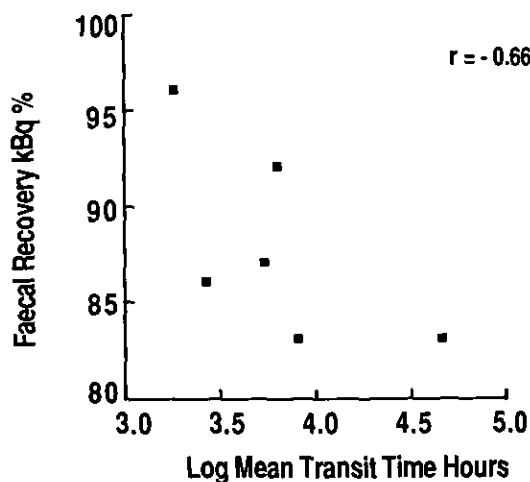


FIGURE 1. Net ^{14}C fecal excretion (percent of administered dose in microcapsules) in relation to transit time of radio-opaque markers (hours).

tured by an ultrasonicator with Ti probe (four 15-sec periods with ice cooling), as reported previously (8). From the resulting suspension, a 100- μL aliquot was taken to measure total radioactivity (core and membrane) and the remainder centrifuged to separate for ^{14}C counting the core (supernatant) and membrane (pellet) fractions. The extent of cross-linking of microcapsule core PEI to membrane PEI by endogenous agents was assessed from alteration relative to an aliquot of unused (control) microcapsules of the ratio:

$$\frac{(\text{membrane } ^{14}\text{C})}{(\text{core } ^{14}\text{C} + \text{membrane } ^{14}\text{C})}$$

To assess ^{14}C loss of radioactivity, ethanol suspensions of microcapsules removed from feces were diluted with water and aliquots taken for Coulter counter assay and scintillation counting in Aquasol, so as to obtain their specific activity (dpm/million). Results were compared with unused (control) microcapsules.

Results

Complete recovery of ROM was found from each subject ($99 \pm 1\%$; range 196–200). The mean transit time of ROM through the gut (MTT) ranged from 26 hr in subject 5 to 106 hr in subject 3, and the radioactivity in individual fecal samples correlated well ($r = 0.96$) with the number of ROM recovered. In the last fecal sample from each volunteer (usually day 10), the measured radioactivity was at the baseline levels of the preadministration control fecal sample.

Recovery of radioactivity in the feces was $88 \pm 5\%$ of the administered dose and varied between subjects. The recovery of ^{14}C dose in feces was inversely related to MTT through the gut ($r = -0.66$; Fig. 1). Eighteen out of 20 samples were complete by PABA analysis and there was no detectable ^{14}C in urine.

Microcapsules recovered from human feces and counted by scintillation counting showed significant removal of [^{14}C]CH₃ label, ranging between 29 and 81%. Label cleavage from micro-

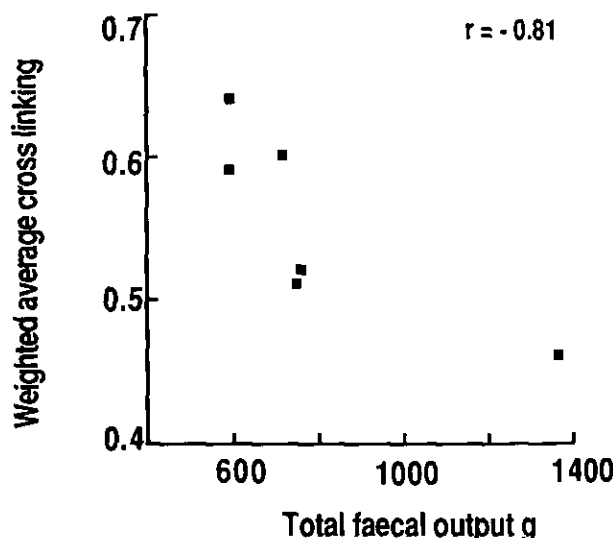


FIGURE 2. Cross-linking in microcapsules in relation to total fecal output during the period of microcapsule excretion.

capsules was unrelated to the total ^{14}C fecal recovery ($r = 0.23$) and to MTT ($r = 0.19$). Comparison of losses for microcapsules on several consecutive days of excretion did not reveal any systematic increase of label loss with longer GI residence. There was no effect of length of time of GI residence on the size of microcapsules recovered from consecutive fecal samples.

In addition, the ratio (membrane ^{14}C /total ^{14}C) was increased more than 5-fold (from 6.8 to <35), i.e., at least one-third of core PEI in the microcapsules had become cross-linked to the membrane by endogenous agents after transit through the gut. After correcting the cross-linking calculation for label loss in each specimen and correcting for the number of microcapsules recovered in each fecal specimen, cross-linking was found to be significantly and inversely related to stool weight, ($r = -0.78$) and to total fecal output ($r = -0.81$) (Fig. 2). There were no significant associations between cross-linking and transit time, nor between label loss and any colonic factor.

Discussion

There has been no previous method for monitoring reactive substances in the lower GI tract where unique biological conditions pertain, and it is perhaps not surprising that novel results were obtained from this first use of microcapsules. It is shown that these microcapsules can be passed through the human GI tract with a similar transit time to inert radio-opaque markers. The unchanged size distribution and lack of effect of gut residence time on microcapsule size distribution in successive stools are consistent with there being no preferential GI retention of smaller microcapsules.

The findings of substantial cleavage of covalently bound label from all microcapsules recovered from volunteers was surprising. Cleavage is presumed to occur in the colon because anaerobic incubation of microcapsules with mixed human fecal flora also demonstrated a substantial loss of the ^{14}C label (9). Loss of label has not been found in rats fed chow, but has been found in rats fed human diets, high in meat, fat and low in dietary fiber (9). Microcapsules treated with H_2O_2 also lose ^{14}C , exclusively from the core (8). This effect is presumed to arise by $\cdot\text{OH}$ radical hydrogen atom abstraction from the [^{14}C]CH₃ label or PEI

molecule leading to production of formaldehyde (hydrate) or a low molecular weight PEI fragment, which would diffuse out of the microcapsules and be incorporated into gut microfloral metabolism. Such incorporation would account for both the relatively high ^{14}C excretion in feces and correlation with ROM excretion, despite most $[^{14}\text{C}]\text{CH}_3$ label being removed from the microcapsules in male volunteers. The origin of this label loss must be substances of low molecular weight able to pass through the semipermeable membranes of the microcapsules (i.e., not enzymes or bacteria). That the mechanism then involves $\cdot\text{OH}$ radical formation is suggested by the apparent iron-dependent effects in microcapsules of H_2O_2 *in vitro* (8) and by the many known substances undergoing Fenton reaction to produce $\cdot\text{OH}$ radicals. The unique structure of these microcapsules containing finely divided Fe_3O_4 and mobile $[^{14}\text{C}]\text{CH}_3$ -labeled PEI chains in close proximity should provide an especially favorable opportunity for such reactions to occur.

In *in vitro* studies, the endogenous DNA-damaging agents fecapentaene-12 and 4-hydroxynonenal were shown to cause cross-linking in microcapsules (8). The finding of substantial cross-linking after GI transit through humans indicates the presence of endogenous cross-linking agents within the human GI tract. Colon cancer risk is inversely associated with fecal weight in human populations, and there is a significant inverse relationship between cross-linking and fecal bulk found in this study ($r = -0.81$; Fig. 2). This, and the absence of a relation between cross-linking and transit time, would suggest that cross-linking is related to proximity between the microcapsules and suspect cross-linking agents within the gastrointestinal tract, rather than extended length of time of residence within the tract. A similar inverse relationship was found also between benzo[a]pyrene metabolite trapping and fecal bulk in F344 rats (12).

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